Annealing:

	Kinased Oligo 2680	Kinased oligo 2723	Control
	1µl	1µl	—
Ann. Buffer	1µl	1µl	1µl
U-ss DNA (gene cleaved)	1.5µl	1.5µl	1.5µl
H_2O	6.5µl	6.5µl	7.5µl.

Anneal at 70°c for 2min → let cool to ~35°c over 30-40 min.

Synthesis:

Add to tubes. 1µl 10x Syn. Buffer
0.3µl T7 DNA Pol (USB)
1.0µl T4 ligase (LTI)

5' on ice, 5' at room temp — 45 min at 37°c.

Add 90µl TE (10mM Tris pH8.0, 0.1mM EDTA).

Transfect DH5αF'IQ.

Transform DH5αF'IQ 100µl with 1.5µl ligation/synthesis mix.
30 min on ice
42°c for 40 Sec. → Split 10% and 90% for 2680 and 2723
Keep 100% for the control.
Add 60µl lawn cells + 3ml soft agar
- let the agar solidified
→ Incubate plates at 37°c.

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ssed & Understood by me,	Date.	Invented by	Date
		Recorded by	